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Occurrence of foodborne pathogen in some types of soft cheese in local market

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بسم الله الرحمن الرحيم

قالوا

سبحانك لا علم لنا
إلا ما علمتنا إنك أنت
العليم الحكيم

صدق الله العظيم

سورة البقرة الآية: ٣٢

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Dedications

My Father

My Mother

My Sister

My Brother

My Uncles

My Family

Always all support religious forever

VITA

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INTRODUCTION

1. INTRODUCTION

E. coli is one of the major significant bacteria, which has a bad effect on both human and animal species. Thus, this type of bacteria can deteriorate the milk particularly raw milk and other milk products as a result of poor hygienic measures (**Garbage *et al*., 2016; Lara *et al*., 2016).**

In Egypt Moreover, Kariesh cheese is a prevalent type of cheese that contains a higher protein content with a small number of fats (**Hamad, 2015).**

Damiati cheese is one of soft pickled cheese and makes up about 75% of the cheese produced and consumed due to its nutritional value, convenience and have a rich taste, strong sharp flavor as well as smooth body and texture.

Tallaga cheese is the most popular local type of packaged or unpackaged soft cheeses by all socioeconomic classes in Egypt due to its nutritional values, conveniences, and good tastes, *E. coli* is classified into six pathotypes: enteroaggregative, enterohemorrhagic Shiga toxin-producing *E. coli* (STEC), enteron invasive, enteropathogenic, enterotoxigenic, and diffuse adherent (**Jafari *et al*., 2012).**

Enteropathogenic *E. coli* (EPEC) is considered the most common bacteria isolated from cheese. EPEC also causes high morbidity and mortality rates among young and old people (**Kostas *et al*., 2010**).

In humans, the pathogenic effect of STEC nearly due to its ability to produce certain types of cytotoxins for example Shiga toxins (*stx1*), and intimin (*eae A*) virulent genes (**Slaney *et al*., 2009 and Assupol *et al*., 2015).**

Shiga toxins–producing *E. coli* (STEC), also known as neurotoxins-producing *E. coli* (VTEC) or Enterohaemorrhagic *E. coli* (EHEC), have been known as a group of highly pathogenic *E. coli* strains producing one or more Shiga toxins (**Monaghan *et al*., 2011).**

Escherichia coli (*E. coli*) is one of the normal inhabitant microorganisms of the large intestine in human and warm-blooded animals. The main source of *E. coli* in raw milk and milk products is fecal contamination together with poor hygienic practices **(Garbage *et al.*, 2016; Lara *et al.*, 2016).** However, certain serotypes of *E. coli* have acquired some virulence-associated genes that enable them to cause intestinal or Extra-intestinal diseases **(Hodson *et al.*, 2017).**

These serotypes that cause enteric infections are generally called diarrheagenic *E. coli* strains, and their pathogenesis is associated with a number of virulence attributes, which vary according to pathotype.

As soft cheeses (Kariesh, Tallaga, and Damietta) collected from different localities from the Gharbia governorate may be subjected to contaminations with *E. coli* and may constitute a public hazard. Therefore, this work was planned to covers the followings points:

1) Isolations and serologically identifications of pathologically pathotypes of Escherichia coli from Kariesh, Tillage, and Damietta types of cheeses in El _Gharbia governorate.

2) Molecular identifications of isolated bacteria (Bacterium Coli) using PCR Techniques and determination of some virulence genes from the isolated strains.

REVIEW OF LITERATURES

2- Review of literature

1.2 .incidence of E.coli in soft cheese

Alice *et al.* (2000) isolated *E. coli* colonies from 44 soft white cheese samples and EPEC serogroups O127 and O128 were found in 11.3% of cheese samples.

El-kosi (2001) examined bacteriologically the kareish cheese samples and *E. coli* recovered as 100 % of all samples.

Menendez *et al.* (2001) examined 24 of kareish cheese samples produced in Galicia. NW Spain for their microbiological characteristics. They found that *E. coli*.

Al – Jeddah and Robinson (2001) examined microbiologically white brined imported Damietta cheese samples from Egypt and purchased from food stores around Doha (Qatar) and found that all samples were free from *E. coli*.

Ahmed (2002*)* examined 20 samples of cheese for the presence *E. coli* and found that 25 % of the examined samples were contaminated with *E. coli*.

Gomez *et al.* (2002*)* examined bacteriologically 114 samples of soft cheese for the presence of *E. coli* and founded 0.9 % of samples were positive.

Amer and Ewin (2003) examined 40 random samples of street vendors kareish cheese collected from different localities in Alexandria and Kufr El-Sheikh governorates. *E. coli* could be isolated from 37.5% of the examined samples.

Thabit. S (2003) examined 40 samples of cheese for the presence of *E. coli* and found that 20% of the examined samples were contaminated with *E. coli*. the isolated strains were serotyped as O111, O119 and O157.

Spano *et al.* (2003) studied experimentally have already shown that treatment at high temperature such as curd stretching at 80 °C for 5 min during

the manufacture of cow's milk mozzarella cheese has been shown to effectively control STEC strain growth

El-Bakery (2004) examined bacteriologically 50 samples of Kareish cheese collected from different markets and shops from Giza, Egypt in summer and winter. He found that the percentage of *E. coli* in Kareish cheese was detected in 18 % and 26 in winter and summer respectively.

Mustafa (2004) found that 30.9% of the examined Damietta cheese samples were positive for *E. coli*.

Oxus *et al*. (2004) examined 50 white pickled cheese manufactured from raw milk for the presence of *E. coli O157 which* was detected in 4% of the total cheese samples.

Hunnish *et al.* (2005) reported that the number of cases deaths 13 in Canada with serotype O175: H7 of *E. coli* from ingestion of contaminated un pasteurized Gouda cheese.

Nemur (2005) isolated *E. coli* from Kareish cheese samples by ann incidence of 24% of the examined samples. The isolated serovars of *E. coli* were O111, O55, O126 and O157: H7 with a percentage 25%, 17 %, 33% and 25%, respectively.

Bah out and Mostafa (2006) examined 50 samples of Kareish cheese collected from local markets in Dakhlia province, Egypt *E. coli* were present.

Anand and Gangapur (2006) tested 77 samples of domestic soft cheese which were collected randomly from different groceries and markets in Kerman, Iran and they found that 98.70% of cheese samples were positive for *E. coli* and Eight *EPEC* serogroups were isolated from 15 (19.48%) of the samples.

EPEC serogroups included O26 (I), O86 (II), O114 (IV), O142 (IV and I), O119 (II), O127 (II), and O128 (III).

El-Pessary (2006) examined microbiologically Kareish cheese samples and pointed out those coliforms, fecal coliforms and *E. coli* were detected in 100%, 92.5% and 60% of the examined samples, respectively.

El-Pessary (2006) examined microbiologically Damietta cheese samples and pointed out *E. coli* were detected in 10% of the examined samples, respectively.

Mahi, Al-Ashmawy (2006) examined bacteriologically 25 samples of Kareish cheese collected from the El-Dahlia governorate for the presence of Coliforms and reported that coliforms were detected in all examined samples.

Johnson *et al.* (2006) found that *E. coli* causes illness ranging from gastrointestinal tract-related complications such as diarrhea, dysentery, urinary tract infection, pneumonia and even meningitis.

Anand and Gangapur (2006) tested 77 samples of domestic soft cheese which were collected randomly from different groceries and markets in Kerman, Iran and they found that 98.70% of cheese samples were positive for *E. coli* and Eight *EPEC* serogroups were isolated from 15 (19.48%) of the samples. EPEC servo groups included O26(I), O86(II), O114(IV), O142 (IV and I), O119 (II), O127(II), and O128(III).

Control and Prevention (206) weekly report which reported that during August-September 2005 a total of 52 (2.44%) of 2,160 inmates at a state correctional facility reported diarrhea, including 17 (33%) with bloody diarrhea. Sixteen of the samples were positive for the *Stx*1 gene but not for STEC O157 Serotyping, and in this case, the source of the Outbreaks likely was an ill food worker.

Laden and raze (2006) examined Seventy-seven random samples of domestic Iranian soft cheese that were collected from different groceries and markets in sterile conditions, cultured in selective media ., and examined for

biochemical tests. Among the 77 samples, *E .coli* was isolated from 76 (98.70%) of them and 15 (19.48%) of the isolates were EPEC. Eight EPEC identified serogroups were included: O26(I), O86(II), O114(IV), O142 (IV and I), O119(II), O128(III), and O127(II). All these types are pathogens. O127 was the most prevalent serogroup, followed by O128 and O119. The occurrence of a high proportion of *E. coli* in our cheese samples may be due to a lack of proper sanitation and the absence of pasteurization of milk used for cheese making. Therefore, strict hygienic measures must be followed and pasteurization of milk should be imposed to prevent contamination of cheese with coliforms, thus avoiding additional outbreaks of food-borne illness caused by *E. coli*.

Abraham *et al.* (2007) examined 80 samples of Kareish cheese collected from the Alexandria government. The prevalence of *E. coli* reached 75%, respectively.

Almeida *et al.* (2007) stated that out of 70 cheese samples were examined for the presence of *E.coli*, O157 for grading them as satisfactory, acceptable, unsatisfactory, or unacceptable and potentially hazardous; 22 were graded as satisfactory or acceptable, 37 were considered unsatisfactory because of the presence of *E.coli*, while 11 of the cheeses samples were graded as unacceptable and potentially hazardous because of the presence of excessive numbers of *E. coli*, in three of these samples.

Paneto *et al.* (2007) collected 50 samples of raw milk cheese from different supermarkets in Middle Western Brazil and were analyzed for *E. coli* which were recovered from 48 (96.0%) of the samples. The identified Serogroups were O125 (6.0%), O111 (4.0%), O55 (2.0%) and O119 (2.0%). Three (6.0%) and 1(2.0%) of *E. coli* isolates were verotoxigenic *E. coli* (VTEC) and enterotoxigenic *E. coli* (ETEC), respectively.

***Om*er *et al.* (2007)** analyzed 80 samples of kareish cheese collected from supermarkets shops and street vendors in Alexandria, Egypt for bacteriological

quality. The examined samples showed that *E. coli* was detected in 60 (75%) in Kareish cheese.

El-baradari e*t al.* (2007) examined microbiologically Damietta cheese samples and they found that 90% of the samples were positive for *E. coli*.

Nagaina and Chen (2008) Reported an association between virulence factors and antimicrobial resistance in *E. coli* strains isolated from dairy cows.

Osman and Omer (2008) found that *E. coli* was detected in some of the cheese samples.

Hassan (2008) reported that microbiological quality, composition, and some chemical properties were determined in 40 samples of Kareish cheese randomly collected from various markets located in Cairo. microbiological examination revealed that 12% of the examined kariesh cheese was contaminated with *E. coli.*

Sabry and Laila et *al*. (2008) examined bacteriologically a total of fifty raw milk and twenty-three Kareish cheese samples were randomly collected from different shops and distributors in Qena city *E .coli* were recovered from 11 (47.8%) of Kareish cheese samples.

Fadel *et al. (*2009*)* examined microbiologically 40 samples of Kareish cheese collected from supermarkets and dairy shops and street vendors in Ismailia city for the presence of *E. coli* and the results were 4 samples only were positive.

Fadel and Jaehn (2009) collected 40 Kareish cheese samples from different localities in Ismailia city and they could isolate 4 different *E. coli* serovars STEC group (O128: H_2), EPEC (O127), EIEC group (O124: H7) , and one untippable strain.

Ali *et al.* (2009) examined 15 random samples of Kareish cheese from dairy shops and street vendors in rural areas for Microbiological evaluation. The incidence of *E. coli* was 6.6% out of the examined samples.

Morass (2009*)* fifty-five samples of Kareish cheese were collected from different non inspected commercial establishments and submitted to the inspection of *E. coli* it was detected.

El-prince, Etnas *et al.* (2010) examined 30 random samples of Kareish cheese that were collected from a suit city, Egypt for fecal pollution indicators. All examined samples were contaminated with coliforms. The incidences of *E. coli* in Kareish cheese were 33.3%.

Kostas *et al.* *(*2010) cheese can be easily contaminated by food handlers who practice poor personal hygiene at some point during cheese preparation and/or assembly line.

Ortolani *et al*. (2010) studied the microbiological quality and safety of 18 soft cheese samples and they found that low levels of *E. coli* were observed.

Todd *et al.* (2010) several foodborne outbreak of colibacillosis investigation reports have identified the hands of food workers as the source of pathogens in the implicated food.

El Sayed *et al.* (2011) examined microbiologically (30 samples of Damietta cheese and 15 samples of Tillage cheese and 10 samples of Kareish cheese) were collected from Cairo and Giza governorates for the presence of *E. coli* and results respectively were (20%, 33,3%, 26.6%).

Ebrahim *Rahimi et al.* (2011) examined microbiologically 120 traditional fresh cheese samples for the presences of *Escherichia coli* in Iran state and the result was four isolates from traditional cheese and the genes *Stx1*

and *Stx2* were detected three *E. coli* isolates obtained from traditional cheeses samples.

Zeinhom (*2011)* tested 50 random samples of Kareish cheese were collected from different localities in Beni -Suef Governorate, Egypt monitoring of enteric pathogen with *E. coli* the data showed that *E. coli* was not isolated from any of the examined samples of Kareish cheese.

Hosni *et al.* (2011) made a survey to evaluate the level of microbial contamination of 35 samples of Kareish cheese retailed in the Cairo area and the results indicated that *E. coli* as a fecal indicator, was isolated from 25.7% of the total numbers of Kareish samples.

Ozan *et al.* (2011) studied the microbiological quality of white pickled cheese produced in small dairy plants which were investigated and found *E. coli* was isolated from 40% of the samples.

Dias *et al.* (2012) reported on the implementation of Good Manufacturing Practices **(GMP)** in a small processing unit of mozzarella cheese in Paraná, Brazil, and that the primary hurdles for the implementation of **GMP** were related to the difficulties to change the hygiene practices of food handlers

Ahmed (2012) examined microbiologically 25 samples of Damietta cheese and 25 samples of Talega cheese and 25 samples of Kareish cheese collected from dairy shops and street vendors in Beni Sufi city for the presence of *E. coli* and the result were 30%, 48%, 27.80%, and 39.94% respectively.

Abd El-Latifa, elan (2012*)* examined bacteriological 25 samples Kareish cheese randomly collected different farmers in El-Bahira, Egypt, and examined microbiologically. The author found that *E. coli* was detected in 44% in Kareish cheese.

Ahmed (2012) examined 25 random samples of Kareish cheese that were collected from different markets and retail shops in Beni -surf city, Egypt

for chemical and microbiological examination. The prevalence rate of *E. coli* isolation was 28.34%.

Arabi *et al.* (2012) investigated the frequency of *Fim H* and other adhesions genes in UPEC and determined the *Fim H* gene frequency as 87.7%.

Brooks *et al.* (2012) obtained 41 raw milk cheese from retail specialty shops, farmers' markets, and on-line sources, and the samples were then analyzed for the presence of *E. coli*. The results revealed that 2 of those contained *E. coli*.

Nagak *et al.* (2012) examined 120 random samples of raw milk, Damietta cheese, Kareish cheese, and Cooking butter (30samples each) which were collected from different localities in Assiut city, Egypt and they found that the incidence rates of *E. coli* in raw milk, Damietta cheese, and Kareish cheese samples were 83 %, 30%, and 60 %, respectively.

Samaha *et al*. (2012) examined 50 Kareish cheese samples that were collected from different localities in Alexandria and the results revealed that the rate of isolation of *E. coli* was 26%.

Clays *et al*. (2013) reported the occurrence of Pathogenic bacteria in raw milk and their products in pathogenic *E. coli,* Therefore, consumption of raw milk and traditional milk products can be caused foodborne pathogens Outbreaks.

Bunyanian *et al.* (2014) examined 50 samples of unpasteurized soft cheeses from retail shops which were analyzed for detection of *E. coli* and found 24 of the cheese samples were positive for *E. coli.*

Gundogan and Arci (2014*)* examined samples of white soft cheese from three different dairy-processing plants in Ankara City (Turkey) to find out if they were contaminated with *E. coli*. The results showed that the contamination percentages of *E. coli* were 60%.

Abo Zed (2014) examined microbiologically 30 samples of Kareish Cheese randomly collected from different dairy shops and supermarkets in Cairo, Giza, and Kilobit governorate, Egypt. The results revealed that *E. coli* failed to be detected.

El- kholy *et al.* (2014) examined bacteriologically 50 Fifty samples of Damietta Cheese were randomly collected from supermarkets, retailers, and shops in the Bani-Suif governorate, Egypt for the presence of *Escherichia coli*, and the result were 13 samples positive by percentage 26%.

Hussain *et al.* (2014*)* collected 120 samples of different dairy products including Cheese from different localities of Lahore and found 54% of tested samples were contaminated with *E. coli*.

Walid *et al.* (2014) examined bacteriologically 40 samples of Damietta Cheese and 20 samples of Kareish Cheese samples for the presence of *Escherichia coli* only 12 samples were positive for *E. coli* presence with 20 %.

Al Jabari *et al.* (2015) examined 70 samples of white Soft Cheese, the results revealed that the incidence of *E. coli* was 87%.

Aseel A. Saeed (2015) examined a total 50 Cheese samples were collected from a market in Al-Diwaniya City In Kuwait the study was done in the period between August and October 2014 and recorded 38 samples were positive for *E. co*li.

Met Wally and Fatma (2015) examined about 25 samples of Kareish Cheese collected from retailers and small dairy shops in Giza and Bani-Suief governorates markets for the presence of *E. coli* and result founded that about 72% of samples were positive for the *E. coli.*

Mohamed (2015) examined Kareish Cheese bacteriologically for the presence of *E. coli* and it is recorded from all samples by a percentage of 100 %.

Elnath *et al.* (2015) examined microbiologically 40 samples of Kareish Cheese samples were collected from different localities in El Monovia - governorate for the presence of *E. coli* and founded 50 % of samples were positive for pathogenic *E. coli.*

Gamal *et al.* (2015) examined microbiologically 50 samples of Damietta Cheese and 30 samples of Kareish cheese were collected from the Cairo governorate for the presence of *E. coli* and the results were 20% and 33% respectively.

El bigotry *et al.* (2015) examined microbiologically 40 samples of Kareish Cheese and 40 samples of Damietta cheese collected from supermarkets and dairy shops and street vendors in El- Gharbia governorate for the presence *E. coli* and the results were 52.5 % and 15% respectively.

Jehan e*t al.* (2015) examined microbiologically 30 samples of Kareish Cheese and 30 samples of Damietta cheese from different shops and supermarkets and street vendors in Ismailia city for the presence of *E. coli* and the results were 9 samples of Kareish cheese were positive and 12 samples of Damietta Cheese were positives.

Abo Baker *et al.* (2016) isolated from fresh locally made Soft Cheeses were 9 (33 %) and 3 (11 %) respectively from (masoor and Ricotta soft cheeses) positives for the presence of *E. coli.*

Gamal and soda (2016) examined 50 samples of Damietta cheese and 50 samples Kareish Cheese in Cairo and Giza governorates for the presence of the *E.coli.*

2.2 .phenotypic character of E.coli

Viipuri *et al.* (2013*)* examined 50 samples of soft cheese for the presence of *E. Coli* and founded that 28% of samples were positive for *E. coli* were collected from milk vendors, retail shops located in Anand city, (India) under aseptic precautions. For the enrichment of the organism from the collected samples, MacConkey broth was used and inoculation was carried out on MacConkey agar and EMB agar. Later on, to confirm the isolates, various biochemical tests such as the IMIS test, Urease test were performed. Evaluation of the antibiotic sensitivity pattern of *E. coli* was assessed by the disk diffusion method. Finally, the *E. coli* isolates were screened for the presence of virulence - associated genes by PCR and founded that 7 isolates of *E. coli* positive for the presence of *Stx1* (8.75 %) only and 5 isolates were positive for The presences of both *Stx1* and *Stx2* and 7 isolates were positive for *Eae A* gene.

Lotfy *et al*. (2017) examined 25 samples of each Kareish cheese and Damiatte cheese collected from Mansoura city for the presence of *E. coli* and founded the results were 32% and 16% by using MacConkey agar medium and 44% and 20 % by using EMB medium.

3.2 .in vitro antimicrobial sensitivity

Cergol-Novella *et al.* (2011) showed that the highest resistance rates of STEC were identified for tetracycline (100%), streptomycin (78%), and trimethoprim-sulfamethoxazole (56%). Furthermore, all strains were harbored *Stx* genes.

Assupol *et al.* (2015) demonstrated that the STEC isolates that carried *Stx1* only showed the highest resistance to Streptomycin

(Pure Negative) (85.6%), followed by Kanamycin (72.2%), and Nalidixic acid (70.0%).

4.2. genotypic detection of virulence genes of E. coli

Pradel *et al.* (2000) reported the isolation of STEC non-O157 strains from cheese in France, all of them carrying the *stx*1 gene but not the *eae A* gene.

Rey *et al*. (2006) showed that 3 STEC carried the *Stx1* gene, 5 possessed the *Stx2* gene, and 1 both *stx1* and *Stx2*. Whereas only O157: H7 serotype showed *eae A* virulence gene.

Lothar Boutin *et al.* (2007) examined 219 Shiga toxins producing *Escherichia coli* (STEC) strains from meat, milk, and cheeses samples collected in Germany between 2005 and 2006 and they found that Shiga toxin *Stx1* present in 88 (40 %) strains.

Garofalo *et al.* (2007) studied 18 UPEC isolates collected from females and found that the *Fim H* gene was the most prevalent virulence factor and 100% of the isolates had that gene.

Irion *et al. (*2007) it is also important to mention that in Brazil, human STEC infections have been primarily associated with diarrhea and most of the STEC strains isolated are non-O157 strains harboring *Stx*1 and *Eae A* genes.

Malden *et al. (*2009) evaluated the distribution of virulence genes among the studied UPEC and showed that the prevalence of different virulence genes varied from 10% for the CNF colony necrotizing factor gene to 80% for the *Fim H* gene**.**

Watts *et al.* (2010) demonstrated that the *Fim H* gene was the most frequent virulence gene and was detected in 98% of *E. coli* strains isolated from patients with UTIs.

Shaath Thanom Ahmed *et al.* (2011) examined microbiologically 115 samples of white cheeses from 3 different areas in Baghdad surveyed and examined for the presence of *Escherichia coli* the result showed 7 samples are

positive for the presence of *Escherichia coli* and one strain contain *Stx1* virulence genes.

Mohammed Elhadidy and Mahmoud hammed (2012) examined microbiologically 124 raw milk cheese samples (64 Kareish and 60 Damietta cheese samples) for the presences of STEC using molecular detection of virulence markers such as Shiga toxin *(Stx1)* and intimin gene *(Eae A) not far enough* and by Serotyping they found only 14 samples positive for the *E .coli*.

Elhadidy and Mohammed (2013) studied microbiologically 124 raw milk cheese samples (Kareish and Damietta cheese samples) for *E. coli* contamination. They found that 14 *E. coli* strains were positive for STEC. **Elhadidy and Mohammed (2013)** reported that STEC incidence in 124 cheese samples was 11.29%. All recovered isolates harbored the *Stx2* gene 100% either single or in association with the *Stx1* gene, while *Stx1* was present in 64.28% of isolates. They reported that theintimin gene (*Eae A*) was present in 21% of isolates.

Tarkhun *et al.* (2013) reported that among the studied virulence genes of UPEC strains, the *Fim H* gene was the most prevalent virulence gene and was founded in 68% (61/90) of the UTI isolates.

Durra & Marin (2014) examined 330 *E. coli* strains isolated from 111 Minas Fresca cheeses from Southwest Minas Gerais State and also did not detect *Stx* 1, *Stx* 2, or *Eae A* genes among the *E. coli* isolates.

Hoffmann *et al.* (2014) analyzed 87 *E. coli* strains isolated from pasteurized milk obtained from 22 farm dairies located at Parana state in Brazil and did not detect the *Stx*1, *Stx*2, and *Eae A* virulence genes among the *E. coli* isolates.

Assupol *et al. (*2015) reported from 561 *E. coli* isolates, 90 (16.0%) were carriers of *Stx1*, 97 (17.3%) of *Stx2*, and 45 (8.0%) of *Eae A* genes singly. 37

(6.6%) isolates were carriers of *Stx1* and *Stx2*, 110 (19.6%) were carriers of *stx1* and *Eae A*, and 67 (11.9%) were carriers of *Stx2* and *Eae A*.

Zahren Hejazi *et al.* (2015) a total of 140 isolated *E. coli* strains from patients with UTI were identified using biochemical tests and then evaluated for the *Fim H* gene by polymerase chain reaction (PCR) analysis. The *Fim H* gene was found in 130 isolates (92.8%) of the UPEC strains (How Many Isolates and strains There are big Differences). 130 isolates positives for the *Fim H* gene, 62 (47.7%), and 68 (52.3%) belonged to hospitalized patients and outpatients, respectively.

The results of this study indicated that more than 90% of *E. coli* isolates harbored the *Fim H* gene . The high binding ability of *Fim H* could result in the increased pathogenicity of *E. coli*; thus, *Fim H* could be used as a possible diagnostic marker and/or vaccine candidate.

Mubarak *et al.* (2016) examined 55 Kareish Cheese samples for the presence of *E. coli* and found that 41 samples (74.4%) were positives for *E. coli* Occurrences and these isolates screened by PCR for the presences of virulence genes *Stx1* and *Eae A* virulence genes and the percentages were (90 %), (45%) respectively.

coli and they founded that the results were negatives.

Nabil *et al.* (2016) examined 95 food and environmental samples were examined for the presence of Shiga Toxigenic *Escherichia coli* for the presences of *Stx1* and founded one sample positive for the presence of STEC.

Same a wad (2016) examined microbiologically 15 samples of traditional Kareish Cheese made using raw milk were collected from different markets in different governorates (Alexandria, Behera, Kefir el- Shiekh, Damietta, Gharbia) for the presences of *E. coli* and the results were negatives.

Furthermore, the results showed that 11 out of 11 (100%) *E. coli* had both *Stx1* and *Stx2* while *Eae A* gene was not found in *E. coli* isolated from traditional milk products. The *Eae A* gene is an accessory virulence factor for STEC that is thought to enhance the virulence of STEC, while some STEC strains not harboring *Eae A* gene have been shown to cause human illnesses.

Cholesteric *et al.* (2017) examined microbiologically 26 Kind Cheeses samples randomly purchased from local markets in Kashan, Iran and evaluated for the presence and Occurrence of STEC by culturing and Molecular methods and the incidence of *E. coli* in 26 samples was as follow 11,54 % furthermore isolates were assayed for the presences of Shiga toxins *Stx1* and intimin gene *eae A* . the findings showed that 100 % of *E. coli* contain *Stx1* while *Eae A* gene was no found in *E. coli* isolates of traditional milk products In recent years, Shiga toxigenic *E. coli* (STEC) strains as emerging groups of foodborne pathogens are responsible for most foodborne illnesses. The pathogenicity of these strains is almost related to the ability of the microorganism to produce the one or two cytotoxins called Shiga toxins (*Stx1* and *Stx2*), and the presence of the intimin (*Eae A*) gene, which is responsible for intimate attachment of the organism to the intestinal epithelium.

Douellou *et al.* (2017) demonstrated that the virulence genes profiles of dairy products and human STEC strains were similar to **Farhad shaft chaleshtori *et al.* (2017)**.

Patricia Cardoza and Jose miacid marine (2017) Examined 59 raw milk Cheese samples collected from local producers in Brazil for microbiological analysis and found that 38 (64,4%) tested positive for *E. coli.* then 147 strains of

E. coli from positives samples and screened by polymerases chains reactions for the presence of virulence genes *Stx1* (verotoxin type 1) and *Eae A* (intimin) gene and found that (9,5 %) were positives for the presence the *Stx1* gene and (11) of

them also harbored *Eae A* gene. all STEC – contaminated food can be considered potentially hazardous.

Hussein *et al.* (2019), inspected 75 samples of Kareish Cheese collected from different areas presented in Monofia- governorate According to the results, the frequency of *E. coli* isolated from Kareish was 16%. Serological identification classified the *E. coli* strains into three groups: Enterohemorrhagic *E. coli* (EHEC) serogroups (O26: H11, O91: H21, O111: H2 ,and O103: H2) While the Enterotoxigenic *E. coli* (ETEC) Serogroups were detected as O125: H21 which is the most prevalent strain. O171: H2, O86, and O119: H6 belonging to Enteropathogenic *E. coli* (EPEC). The most prevalent genes detected in *E. coli* strains were *Stx1* (87.5), *Fim H* (75), and *Eae A* (25%) genes. In conclusion, Kareish Cheese is extremely tainted with pathogenic *E. coli* strains, which represents a strong hazard on the Animals and Human health.

Taha *et al.* (2019) examined microbiologically 90 Cheese samples high salt soft cheese, Kareish Cheese, and Talega cheeses were examined for *E. coli*. Serodiagnosis of *E. coli* has been done by slide agglutination test. Toxigenic *E. coli* isolates were detected using PCR assay. Results postulated that the detection rate of non-O 157 was 36,33% in Kareish Cheeses, 20 % in high salt soft Cheeses, and 33,33% in Talega cheeses. Ten different serotypes of *E. coli* non O157 Serotypes *of E. coli* non-O 157 have been identified as follows: O1, O18, O20, O25, O26, O125, O126, O127, and untyped *E. coli*. regarding PCR results: all serogroup was negative for the *Stx1* gene, only servo group 25 positives for *Eae A* gene.

Ibrahim *et al*. (2019) examined bacteriologically 30 samples of Damietta cheese and 30 samples of Kareish Cheeses randomly collected from local markets, farmers vendors, and supermarkets at different localities in the Nile Delta region, Minufia- governorate, Egypt. *E. coli* recovered from samples were 13 (43,3 %) and 22 (73.3 %) respectively. The Serotyping reveals in Kareish

Cheeses as following O128; H2, O26; H11, O44: H 18, O119: H6, O127: H6, O127: H6, O111: H2, O124, O146: H21 and in Damietta cheese as following :O128: H2, O26: H11, O91: H21, O127: H6 , O111: H2, O124, O146 H41and PCR results as following: O26; H11 had *Stx1* and *Eae A* genes, O44: H 18 had not S*tx1* and *Eae A* gens, O91: H21 had *stx1* and had not *Eae A*, O111: H2 had *stx1* and *Eae A* genes, O124 had not *stx1* and *Eae A* genes.

5.2. public health importance

Hussein and Sakuma (2005) studied the role of dairy cattle and their products in human infections with Shiga toxin-producing *Escherichia coli* (STEC). Shiga toxin-producing *E. coli* were isolated from raw milk, milk filters, and dairy products such as yogurt and soft cheeses. STEC infection was attributed to the consumption of dairy products that were contaminated with cattle feces. Thus, dairy cattle are considered reservoirs of STEC and can impose a significant health risk to humans.

Espies *et al.* (2006) reported that there were many cases of deaths in France due to the contamination of fresh unpasteurized goats' cheese by *E. coli* serotype O157: H7**.**

Ibrahim *et al.* *et al.* (2020) examined non-O 157:H 7 Shiga-toxin producing *Escherichia coli* (STEC) is a major foodborne pathogen that causes severe disease in humans ranged from mild gastroenteritis to critical illness and death especially O 26. O 111 in kareish cheeses**.**

MATERIALS AND METHODS

3-Materials and Methods

1. Materials

3.1. Samples:

One Hundred and fifty random samples of soft cheeses (50 samples of Kariesh Cheese – 50 Samples Of Tallaga Cheese – 50 Samples Of Domiatti Cheese) Were Collected Randomly From Small Diaries, Local Super Markets, Farmers Houses, Shops ,And Street Vendors In Different Localities At Gharbia Governorate. The Collected samples were transferred directly to the laboratory in an ice – box under aseptic conditions without delay and then subjected to bacteriological examination.

3.2.1. Isolations of *E*. coli:

5 grams of samples of each (Kariesh Samples – Tallaga Cheese- Domiatti Cheese) were added to 25 ml sod. Citrate and blended for two minutes for the examination of *E. coli* by these tests. (Preparation of samples): (maintain PH) (flushing)

3-2-1.1- MacConkey's broth test

(Acid And Gas Test): Two ml of each blended samples are added to MacConkey broth with bin Dorf tubes and incubated for24 hours at 37^{o}C for the detection of acid and gas if it is presently indicated for the presence of *E. coli.* (Selective Media is Gram -Negative Facultative Anaerobic Enterobacteriaceae)

3-2-1.2- Eosin Methylene Blue Media Test:

From the positive MacConkey's broth test with yellow colorations and acid and gas results are cultured on the Eosin Methylene Blue agar plates (EMB) is Selective Media for *E. **coli,*** Then Incubated at 37 ºC for 24 hours. Typical colonies of *E. coli* appeared Greenish Metallic with or Without Dark Black Centers. Suspected colonies were picked up and transferred to nutrient agar

slopes and then incubated at 37 ºC for 24 hours. The purified colonies were subjected to Biochemical and Serological Examinations.

3-2-2: - Biochemicals Identifications:

The pure colonies of isolates were identified biochemically as fellow: -

3.2.2.1- Indole Test: (Positive)

To each 48hrs incubated culture at 37ºC in 1% peptone water, one ml of ether was added. The tubes were vigorously mixed and allowed to stand for a few minutes until the ether rises to the surface. About 0.5ml of Kovac's reagent was added on the side of the tube gently. The Development of a rosy color ring indicated the presence of indole.

3.2.2.2- Citrate Utilization Test: (Negative)

The ability to utilize citrate was detected by making a single streak over the surface of Simmons's citrate agar slope, then incubated at 37ºC for 7days. The development of blue coloration indicated citrate utilizations.

3.2.3-Serological Identifications of *E. coli* serovars:

Two separate drops of saline were put on a glass slide and a portion of the colony from the suspected culture was emulsified with the saline solution to give a smooth fairly dense suspension.

- To one suspension, control, one loopful of saline was added and mixed. To the other suspension, one loopful of the undiluted antiserum was added and titled back and forward for one minute.
- Agglutination was observed using indirect lighting over a dark background. When a colony gave a strongly positive agglutination with one of the pools of polyvalent serum, a further portion of it was inoculated onto a nutrient agar slant and incubated at 37C for 24 hours to grow as a culture for testing with monovalent sera.

- A heavy suspension of bacteria from each slope culture was prepared in saline, and slide agglutination tests were performed with the diagnostic sera to identify the O-antigen.

3.2.4-In-Vitro antibiotic sensitivity of E. coli:

Subcultures from the isolates were prepared and the test was applied as follows:

A smooth single colony was inoculated in 5 ml nutrient broth and incubated at 37°C for 18 hrs., then turbidity was adjusted to 0.5 McFarland contain (1.5×10^8) colony-forming unit/ml, then few drops of the inoculated broth were flooded on to the surface of Muller-Hinton agar plates. Excess of cultural fluid was removed aseptically and the plates were allowed to stand for 15 minutes at 37°C for dryness. Then the inoculated plates were overlaid with antibiotic discs using sterile forceps, the discs were distributed in a manner where the distance among them was optimum and away from the edge of the plate to avoid overlapping of inhibition zones and give more wide area for the zone of inhibition. The inoculated plates were incubated at 37°C for 24 hours. The Inhibition zones, in mm, were measured and scored as sensitive, intermediate, and resistant categories with the critical breakpoints recommend via CLSI **(CLSI, 2018).**

3.2.5-Polymerase Chains Reactions Techniques (PCR):

3.2.5.1- Materials used for extractions of DNAs:

3.2.5.1.1-QIAamp DNA Mini Kit-Catalogue no.51304.

The QI amp DNA Mini Kit provides silica-membrane-based nucleic acid purification from different types of samples. The spin-column procedure did not require mechanical homogenization, so the total hands-on preparations time is only 20 minutes.

3.2.5.1.1-Ethanol 96% Apply chem.

3.2.5.2-Equipments and apparatuses used for extractions of nucleic acids:

3.2.5.2.1-Epindorff tubes 1.5 ml capacity.

3.2.5.2.2--Monochannel micropipettes 20-200 µl, 100-1000 µl (Biohit).

3.2.5.2.3--Sterile filter tips. (200 µl,1000 µl) capacity.

3.2.5.2.4-Centrifuge (Sigma sartorius).

3.2.5.2.5-Type II-A biosafety cabinet (Thermo).

2.5.3-PCR Master Mix used for c PCR: -

3.2.5.3.1-Emerald Amp GT PCR master mix (Takara)Code No. RR310AContains:

a) Emerald Amps separates the GT PCR master mix (2x premix).

b) PCR grade water

c) Oligonucleotides primers used in PCR.

They have specific sequences and amplify a specific product as shown in Table (primers-securities imported bio keys).

Table (specific not copied): Oligonucleotide primers sequences

Source: Metabolon (Germany).

Selected V Genes	Sequences	Amplified products	References
St 1	ACACTGGATGATCTCAGTGG	614 bp	Dipineto et al., 2006
	CTGAATCCCCCTCCATTATG		
Fim H	TGCAGAACGGATAAGCCGTGG	508 bp	Ghanbarpour and Salehi, 2010
	GCAGTCACCTGCCCTCCGGTA		
Eae A	ATGCTTAGTGCTGGTTTAGG	248 bp	Bisi-Johnson et al., 2011
	GCCTTCATCATTTCGCTTTC		

3.2.5.4-DNA Molecular weights markers: -

3.2.5.4.1-Gel Pilot 100 bp plus ladder (cat. no. 239045): - supplied from QIA GEN (USA)

A) - Number of bands: 11

B) - Sizes ranges : 100-1500 bp.

C) - Genes rulers : 100 bp DNA ladder (cat. no. SM0243) supplied from ferments.

3.2.5.5-Materials used for agarose gels electrophoresis:

3.2.5.5.1- Agarose 1.5% (Sambrook *et al.*, 1989)

A multi-purpose, high gels strengths agarose is suitable for a wide range of molecular biologics techniques. It has high gel strength and limited differentiated limits, multi Agarose could effectively separate large DNA fragments with reduced running times. This in turn means less band diffusion, a problem often associated with long running times.

It was prepared as follow:

A) - Agarose powders (AP genes): 1.5 g.

B) - TBE : 100 ml.

D) - Ethidium bromide solution 10 mg / ml (Sambrook *et al*., 1989)

i) - Ethidium bromide powders (Sigma) 10 mg.

ii) - Sterile DW 1.0 ml.

It was mixed and stored covered at 4˚C

It was added to melted agarose to reach a final concentration of 0.1-0.5 µg/ml.

C) - Tris borate EDTA (TBE) electrophoresis buffer (1x) (WHO, 2002) :

i) - Tris buffer (Fluka) : 10.78 g.

ii) - Boric acid (Fluka) : 5.5 g.

iii) - EDTA for DNA Fragments easily separations (Winlab) : 0.82 g .

It was brought up to 1 liter with deionized water, pH was checked up. If the pH was out of the range of (8-8.6), new solutions were surely preparing.

Any changes in ions concentrations will affect the migrations of the DNA Fragments through the gel.

3.2.5.6-Equipment and apparatuses used in PCR :-

i)- Calibrated cylinders.

ii)- Glass flasks.

iii)- CR tubes 0.2 ml capacity.

iiii)- (Scales Tec).

iv)- microwave (Panasonic).

v)- mono channels micropipettes (2-20 µl) (Bio hit).

vi)- Sterile filter tips.

vii)- Gel casting apparatus (Biometry).

viii)- T3 Thermal cycler (Biometry).

viiii)- power supply (Biometry).

viiii)- Type II A biosafety cabinet. (Thermo).

x)- Gel documentation system. (Alpha Innu tech).

xi)- Deionizer (Millipore).

xii)- Double distillatory (Sanyo).

3.2.5.7-Method:-

A- Extractions of DNAs according to QI amp DNA mini kits instructions:

1. rpm for 1 min. The QI amp mini spin column was placed in a clean 2 ml collection tube, and the tube containing the filtrate was discarded.
2. The QI amp mini spin column was carefully opened and 500 ml buffer AW1 was added without wetting the rim. The cap was closed, and centrifugated at 8000 rpm for 1 min. The QI amp mini spin column was placed in a clean 2 ml collection tube, and the tube containing the filtrate was discarded.
3. The QI amp mini spin column was carefully opened and 500 ml buffer AW2 we added without wetting the rim. The cap was closed, and centrifugated at full speed for 3 min.
4. The QI amp mini spin column was placed in a new 2 ml collection tube and the old collection tube was discarded with the filtrate. Centrifugation at full speed for 1 min was done.
5. The QI amp mini spin column was placed in a clean 1.5 ml microcentrifuge tube, and the collection tube containing the filtrate was discarded. The QI amp mini spin column was carefully opened and 100 µl buffer AE was added. The QI amp mini spin column was Incubated at room temperature (15-25˚C) for 1 min, and then centrifugated at 8000 rpm for 1 min.

2- Preparations of PCR (s) Master Mix according to Emerald Amp GT PCR master mix (Takara): Code No. RR 310A kit.

Component	Volume/reaction
Emerald Amp GT PCR master mix (2x premix)	12.5µl
PCR grade water	4.5 µl
Forward primer (20 pmol)	1 µl each
Reverse primer (20 pmol)	1 µl each
Template DNA	6 µl
Total	25 µl

3- Cycling conditions of the primers during PCR.

1. The Temperature and time conditions of the two primers during PCR are shown in Table (paid specifics) according to specific authors and Emerald Amp GT PCR master mix (Takara) kit.

Showing Tables: Cycling conditions of the different primers during

PCR Techniques: -

Genes	Primary denaturation	Secondary denaturation	Annealing	Extension	No. of cycles	Final extension
Stx1	94˚C 5 min.	94˚C 30 sec.	58˚C 40 sec.	72˚C 45 sec.	35	72˚C 10 min.
FimH	94˚C 5 min.	94˚C 30 sec.	50˚C 40 sec.	72˚C 45 sec.	35	72˚C 10 min.
EaeA	94˚C 5 min.	94˚C 30 sec.	51˚C 30 sec.	72˚C 30 sec.	35	72˚C 7 min.

4- DNAs Molecular weights markers: -

The ladder was mixed gently by pipetting up and down. 6 µl of the required ladder were directly loaded.

3-5- Agarose gel electrophoreses (Sambrook *et al*., 1989):-

Electrophoresis grade agarose (1.5 g) was prepared in 100 ml TBE buffer in a sterile flask, it was heated in a microwave to dissolve all granules with agitation, and allowed to cool at 70˚C, then 0.5µg/ml ethidium bromide was added and mixed thoroughly.

The warm agarose was poured directly in the gel casting apparatus with the desired comb in the apposition and left at room temperature for polymerization.

The comb was then removed, and the electrophoresis tank was filled with TBE buffer. Twenty µl of each unity plex PCR product and 30 µl of each multiplex PCR product, negative control, and positive control were loaded to the gel. The power supply was 1-5 volts/cm of the tank length. The run was stopped after about 30 min and the gel was transferred to the UV cabinet. The gel was photographed by a gel documentation system and the data we are analyzing's through computers soft wares.

Results

4- RESULTS

The morphological characters of the colonies on Eosin Methylene Blue were a brilliant green. A total of 64 bacterial isolates were obtained from examined samples, 27 Bacterial Isolates From Kareish Cheeses and 21 Bacterial Isolates From Damietta Cheeses And 16 bacterial isolates from Tallaga Cheese *E*. coli Isolated From Kareish Cheeses With An Incidences (54%) *E*. coli isolated from Damietta cheeses with an incidence (42%) *E*. coli isolated from Tallaga cheese with an incidence (32%) as shown in (Table 3).

E. coli gives Positive results to Indole Test results and Simmons citrate Negative.

Sex *E*. coli isolates were serotyped, Five from Kareish Cheeses, and One from Tallaga Cheese, Serological Identification revealed that Five isolates from Kareish Cheeses were belonging to (O119: H7, O111: H8, O86: O18: H1: K7 and O128). One isolates from Tallaga Cheese belonging to (O124).

Table (1): Incidence of *E. coli* isolated from soft cheeses in El-Gharbia - Egypt:

Type Of Sample	Number of The Sample	Number of Positive samples	Percentage of Positive samples
Soft Cheese	150	64	42.5 %
Kariesh Cheese	50	27	54%
Tallage Cheese	50	16	32%
Domiatti Cheese	50	21	42%

Tables 2. Regional Incidence of E.coli in Soft Cheese :

Regions/Kariesh Cheese	Number	Positive Results	Percentage
Kafer EL-Zayate City	10	2	20 %
EL-Santta	10	Zero	Zero
EL-Mahlla EL-Cobra	10	10	100 %
Kottor	10	10	100%
Tanta	10	5	50 %

Regions/DomiattCheese	Number	Positive Results	Percentage
Kafer EL-Zayate City	10	Zero	Zero
EL-Santta	10	10	100 %
EL-Mahalla EL-Cobra	10	5	50%
Kottor	10	5	50 %
Tanta	10	1	10

Regions/ TallagaCheese	Number	Positive Results	Percentage
Kafer EL-Zayate City	10	ZERO	ZERO
EL-Santta	10	ZERO	ZERO
EL-Mahlla EL-Cora	10	4	40%
Kottor	10	7	70 %
Tanta	10	5	50 %

Table (3) Biochemical tests of *E*. coli:

Bacteria	Biochemical test	Biochemical test
	Indole	Citrate utilization
E. coli	+	-

Table (4): Results of serological identification of *E*. coli isolates: -

Serial	Identified Bacterium	Polyvalent	Monovalent	Strain
1	*E*. coli (K3)	1	O119	EPEC
2	*E*. coli (K1)	1	O111	EHEC
3	*E*. coli (K2)	1	O86	EPEC
4	*E*. coli (K5)	3	O18	Ex PEC
5	*E*. coli (K6)	1	O128	EIEC
6	*E*. coli (D3)	7	O124	EPEC

K =Kariesh Cheese, D=Domiatti Cheese , T= Tallaga Cheese.

Table (5): Antimicrobials sensitivity results for *E.* coli isolates according to CLSI (2018):

Antimicrobial agent	Diffusion zone break point (mm)	E.coli D3 O124	E.coli K3O119	E.coli K2 O186	E.coli K1 O111	E.coli K5O18	Sensitivity Percent
Ciprofloxacin (CIP)	12 (mm)	S(20)	S(28)	S(29)	S(27)	S(32)	100%
Enrofloxacin (ENR)	0.5(mm)	S(8)	S(9)	R(0.3)	S(7)	S(8)	83.33%
Chloramphenicol (C)	15(mm)	S(23)	S(26)	R(7)	R(8)	I(17)	59%
Oxytetracycline (OT)	14(mm)	S(22)	S(21)	R(11)	S(25)	S(24)	83.33%
Neomycin (N)	12(mm)	I(14)	R(9)	R(8)	R(9)	R(7)	%8
Nitrofurantoin (F)	32(mm)	R(25)	S(75)	R(24)	R(19)	S(95)	50%
Erythromycin (E)	13(mm)	R(9)	I(17)	R(9)	S(28)	R(7)	25%

S= sensitive R= Resistant I = intermediate

K= Kariesh cheese D=Domiatti cheese

Table (6): Results of molecular identification of *stx1, eae A* ,and *fim H* genes of *E.* coli:

Sample	*eaeA*	*fimH*	*stx1*
1	-	+	-
2	-	+	-
3	+	+	-
4	+	+	-
5	-	+	-
6	-	+	-
7	-	+	-

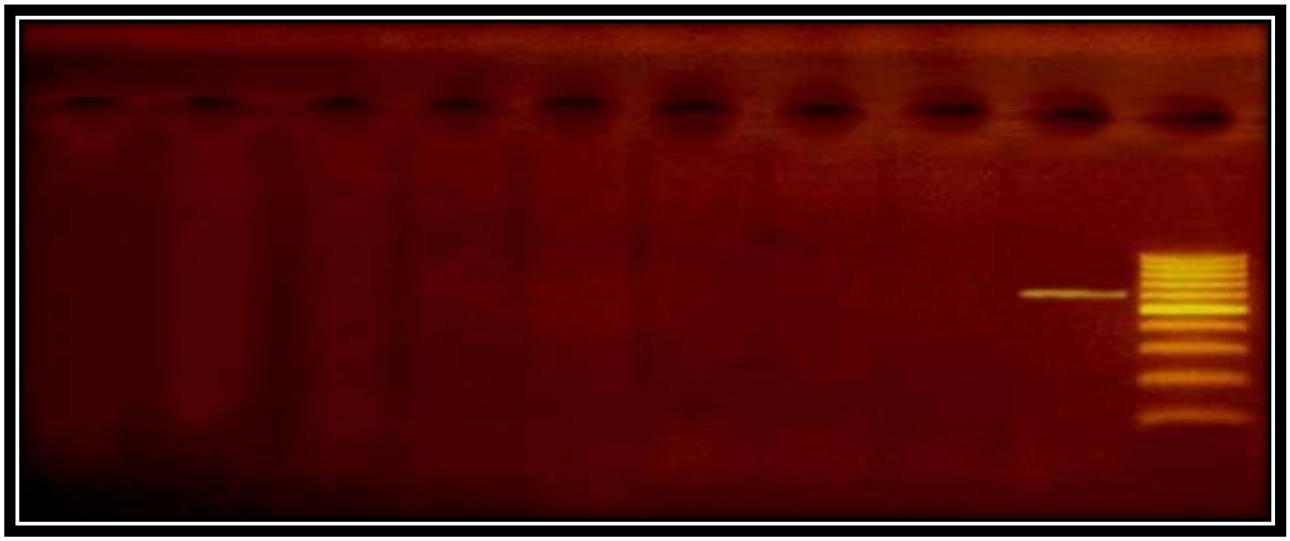

Figure (1)

Agarose gel electrophoresis of PCR amplified products of the virulence gene. For *Stx1* genes were Negatives.

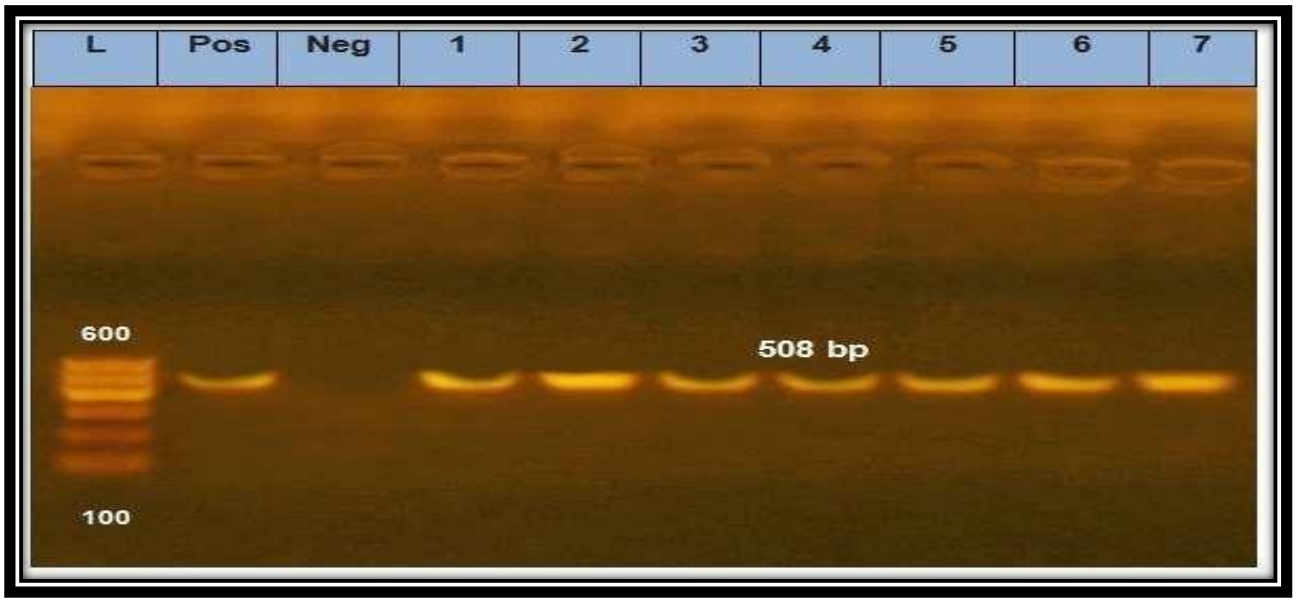

Figure (2)

Agarose gel electrophoresis of PCR amplified products of the virulence gene. Lane L: DNA molecular size marker (100bP), lane Pos: Positive control, lane Neg: Negative control, Lane 1,2,3,4,5,6,7: for detections of *Fim H* virulence gene of *E.* coli. The size in base pairs (508bP) of PCR products is indicated for the bands. All seven isolates were positives.

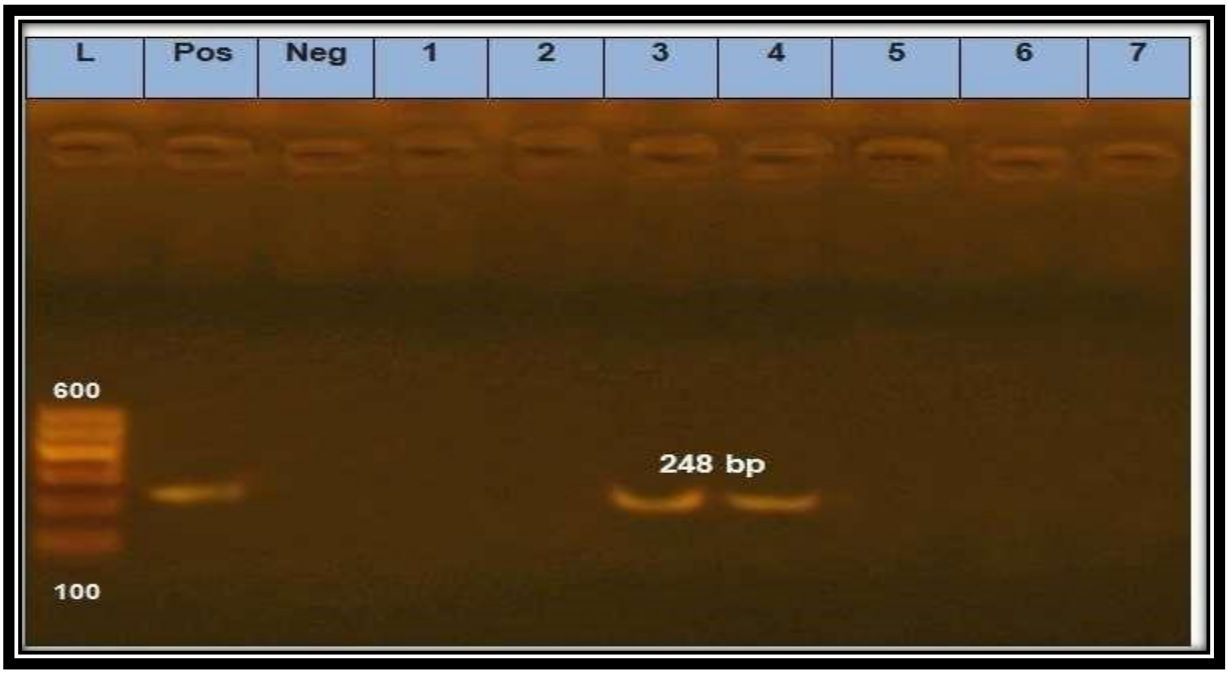

Figure (3)

Agarose gel electrophoresis of PCR amplified products of the virulence gene. Lane L: DNA molecular size marker (100bP), lane Pos: Positive control, lane Neg: Negative control, Lane 2,3: *Eae A* positives virulence genes of *E.* coli. The size in base pairs (248bP) of PCR products is indicated for the bands.

DISCUSSIONS

5- Discussions

E. coli has largely been regarded as an opportunistic pathogen causing both intestinal and extraintestinal diseases associated with large numbers of food poisoning outbreaks **(Varnam and Evans, 1991*).***

Enteropathogenic *E. coli* causes about 30-40 % of infantile diarrhea, as it is the most frequently isolated bacterial pathogen from the cheeses ***(*Doyle and padyhe, 1989)**.

Escherichia coli is commonly present in the intestinal tract of humans and animals, and its detection in processed foods is an indication of fecal matter contamination. It also indicates the inadequate control of the environment of the cheese processing unit, the personal hygiene of the food handlers, and the poor quality of the finished product **(Kostas *et al.*, 2010*;* Dias et *al.*, 2012*).*** Because the occurrence of an outbreak of foodborne disease is caused by enteropathogenic *E. coli* and is associated with the consumption of soft - ripened cheese *(***Maurier *et al.*, 1973),** the presence of these microorganisms in cheese has taken on added significance.

E. coli* is** responsible for several outbreaks of diarrhea in children and adults after ingestion of contaminated milk and dairy products. Different studies showed that 1 - 5% of food-borne infections were related to consumption of milk and dairy products, that 53% of cases of food-borne infections caused by contaminated cheese , and that enteropathogenic ***E. coli (EPEC) is the causative agent of 18.33% of these cases ***(*Schade *and* Yeager, *2001).***

EPEC strains have been implicated in food-borne human illnesses, especially as an important agent of infantile diarrhea in developing countries ***(*Silva *et al.*, 2001)**. EPEC strains are genetically mutative and cause different types of diarrhea in patients. A combination of these strains with

enterohemorrhagic *E. coli* increases their ability to create systematic infections in humans (**Desilk *et al.*, 1991).** The pathogenicity of EPEC strains is not known completely but most of them produce Vero toxins which are different from the enterotoxigenic *E. coli* (ETEC) toxins ***(*Campos *et al*., 1994*;* Crossly *et al.*, 1955).** Some EPEC strains are attached to the intestine epithelium ***(Campos et al.*, 1994).** Different strains of EPEC cause a different level of infection, and it has been shown that these strains are continuously changing and their genome becomes similar to hemorrhagic strains ***(*Campos *et al.*, 1994).**

Coliforms could be found in cheeses and are used as a hygienic indicator for such products. The presence of coliforms in cheese and their relation to enteropathogenic ***E. coli*** in soft cheeses have received considerable attention in previous studies ***(*scared *and* Yeager, 2001; Maurier *et al.*, 1973*).*** The relation between pathogenicity and different serotypes of ***E. coli*** has been suggested and proved ***(*Crossly *et al*., 1995*).***

Contamination of dairy products by EPEC strains has been investigated and O126, O128, O25, and O125 were isolated (**Abbar, 1988; Ahmed *et al.*, 1988*).*** Cheese has been known as an important animal origin product and in most countries, domestic and traditional produced cheese is in demand.

Shiga toxin-producing ***E. coli*** (STEC) are among the most important causes of foodborne diseases. They are responsible for several human gastrointestinal diseases, including watery or bloody diarrhea (**Paton & Paton, 1998).** The primary virulence factor of STEC is the production of common feature and the Shiga toxin 1 (Stx1), Shiga toxin 2 (Stx2) , or its variants, or a combination of these. Highly human-virulent STEC strains are also called enterohemorrhagic ***E. coli*** strains (**Bolton, 2011).**

Food is an important vehicle for the transmission of STEC strains, and most of the foodborne outbreaks caused by STEC have been associated with

unpasteurized milk and its derivatives **(Farrokh *et al.*, 2013).** This study aimed to assess the STEC occurrence in the collected cheeses samples.

Pathogenic STEC strains usually contain other virulence factors such as the outer membrane protein intimin (*Eae* gene), a protein essential for the intimate attachment, and the formation of attaching and effacing lesions on gastrointestinal epithelial cells (**Bolton, 2011*).*** Most outbreaks and sporadic cases of hemorrhagic colitis (HC) and hemolytic uremic syndrome (HUS) have been attributed to strains of serotype O157: H7. However, in some countries, the importance of non-O157 STEC as causes of HUS, HC, and other gastrointestinal disease is being increasingly recognized, particularly the serogroups O26, O103, O111, O121, O45, O145, and O91 **(Farrokh *et al.*, 2013).**

Illness caused by enterohaemorrhagic ***E. coli*** can range from self-limited watery diarrhea to life-threatening manifestations such as hemorrhagic colitis, hemolytic uremic syndrome, thrombocytopenic purpura and may lead to death **(alexander and Prado, 2003)**.

Recently there is additional concern about the presence of *E. coli* in cheeses because of the ability of some strains to cause foodborne illness and lead to a high incidence of food outbreaks **(Chapman, 2000)**.

As milk is of high nutritive value containing vitamins, minerals, and proteins so it promotes the growth of ***E. coli*** and other many microorganisms as a result of contamination from using unsterilized utensils, improper preparation of dairy animals, uses of contaminated water supplies, or careless method of handling **(Trevena *et al.*, 1999)**.

Kareish cheese is considered one of the excellent sources of protein, phosphorus, calcium, and many trace elements and due to its high nutritive value and low prices, it is considered as a popular food in Egypt. It is

manufactured from skim milk naturally fermented by lactic acid bacteria at home, so it may contaminate from sources during is manufacture, handling , and distribution that renders it a source of infection with many diseases **(Thabit, 2003)**.

Therefore, evaluation of the hygienic quality of Kareish cheese manufactured in el Gharbia governorate if one of the objects of this study.

Although milk pasteurization removes almost all pathogenic *E. coli*, inactivation of ***E. coli*** Shiga toxins has not been proven. However, following the consumption of pasteurized milk, an outbreak in North Cumbria, England, during 1999 with HUS cases have been reported and no living bacteria were found in the milk samples **(Goh *et al*., 2002).**

Epidemiological studies suggest that nonO157 Shiga toxin-producing ***E. coli*** (STEC) is a major player associated with foodborne disease outbreaks **(Johnson *et al*., 2006).**

The ten most clinically relevant STECs belong to serogroups O26, O103, O111, O145, O157, O91, O113, O128, O45, and O121 have been reported in milk and dairy products causing severe illness in humans including diarrhea, hemorrhagic colitis, and hemolytic uremic syndrome and may result in death (**Farrokh *et al*.,2003).**

Karish cheese serves as an ideal medium for STEC bacterial growth due to the synergistic effect of different factors that exist in cheese as (low pH, water activity, NaCl, and starter culture) **(Galvez *et al*., 2007)**.

In this work the detection of ***E. coli*** in soft cheeses was performed by isolation and identification of **Enteropathogenic *E. coli*** strains serotypes.

In the present study a total number of 150 samples of Karish cheese, Tallaga cheese, Domiatte cheese (50 samples of each) were collected from different localities in el – Gharbia governorate, Egypt, and examined for the

presence of *E-coli.* The Tallaga and Domiatte cheese from cheese dairy shops and Kareish cheese from both dairy shops and street vendors.

The incidence of ***E-coli*** in Kareisg cheese and Domiatte Cheese and Tallaga cheese proved by Selective culturing on (Eosin Methylene blue media) were isolated and identified as ***E –Coli*** as fellow: Table (3)

1- Kareish cheese:

Inspection of the table before revealed that *E. coli* could be isolated from examined Kariesh Cheese samples with an incidence rate of 54%. A nearly similar finding was reported by El-bajory *et al.*, (2015), El- nahas., *et al* (2015), Nagah *et al.*, (2012). a higher incidence was reported by Ibrahim *et al.*, (2019), Ombark *et al.*, (2016), El-kosi (2001), Mohammed (2015) while the lower incidence was reported by El-Sayed *et al.*, (2011), Zeinhom (2011), Hosney *et al.*, (2011), Hefner (2012), Abd EL- Latif, Ahmed (2012), Brooks, *et al.*, (2012), Nagah *et al.*, (2012), Samaha *et al.*, (2012), Elhadidy and Mohammed (2013), Bonyadian *et al.*, (2014), Abo Zeed (2014), Metwally and Fatma (2015), El Nahas *et al.*, (2015), Gamal *et al.*, (2 015),

Jehan *et al.*, (2015), Gamal and Soad (2016), Sameh (2016) and

Lotfy *et al.*, (2017).

2-Domiatti cheese:

It is evident from the table before that ***E. coli*** could be isolated from examined Damietta cheese samples with an incidence rate of 42%. A nearly similar finding was reported by **Ozen *et al.*, (2011), Ibrahim *et al.*, (2019) while the lower incidence was reported by Zeinhom (2011), Hefney (2012), Nagah *et al.*, (2012), Elhadidy and Mohammed (2013), Gamal and Soad (2016), Jehan *et al.*, (2015), Gamal *et al.*, (2015), Elbassary (2006), El-bajory ,*et al.*, (2015) and Lotfy *et al.*, (2017).**

3-Tallaga cheese:

It is evident from the table before that ***E. coli*** could be isolated from examined Tallaga cheeses samples with an incidence rate of 42%.

A nearly similar finding was reported by **El-Sayed *et al.*, (2011),** while alower incidence was reported by**, Hefney (2012),** Contamination of milk and milk products, with ***E. coli*** is largely due to unhygienic condition during production, processing, handling, and distribution. ***E. coli*** is a good indicator of fecal pollution as it exists in the normal microflora of the intestinal tract of humans and warm-blooded animals **(Olfa *et al.*, 2013).**

2-Serological typing of isolated *E. coli* strains: Table (4)

It is evident from the results recorded in a table (5) that some of the isolated ***E. coli*** strains (64 strains) could be serologically typed into; Enteropathogenic (EPEC), Enterohemorrhagic (EHEC), Enteroinvasive (EAEC) included O119, O111, O86, O18, O128, O124**.**

The Serologically identified pathotypes of *E. coli isolates* from some types of soft cheese were:

(O111) **(EPEC)** Enteropathogenic ***Escherichia coli***, (O86) (**EHEC)** Enterohemorrhagic ***Escherichia col*i**, (O 18) (**Ex PEC)** extraintestinal pathogenic *Escherichia coli*, **(O128) (Atypical EPEC) Atypical EPEC** *Escherichia coli,* (**O124) (EIEC**) Entero Invasive ***Escherichia coli***. ***E. coli*** infections from contaminated cheeses surely constitute the publics hazards of living creatures especially in humans like meningoencephalitis, pyelonephritis, hepatitis, pneumonitis, pleurisy, pericarditis, peritonitis, osteomyelitis, appendicitis.

And even causes outbreaks and death of the consumers because cheeses eating without further heating for pasteurizations.

In this study, ***E .coli*** was isolated from 43% of samples, and 75% of isolates belonged to the EPEC serogroup. Recent studies in other countries indicate similar results. **Araujo *et al., (2002)*** detected high levels of fecal contamination in 95.5% of cheese samples in Brazil, and EPEC was isolated from 21.1% of the samples. In Egypt, 60 Karih cheese samples were examined **and *E. coli*** was isolated from 75% of the samples. Similarly, **Abbar and Kadder (1991**) reported that 40.5% of cheese samples in Iraq were contaminated with EPEC strains. In the present study, we detected 6 different EPEC serogroups in Egyptian soft cheese and that O127 was not found serogroup. Recent studies in other parts of the world indicate different prevalent EPEC serogroups in soft cheeses. In Brazil, O127 was the most frequently found serogroup, followed by O55 and O26 (**Araujo et al., 2002). Ahmed et al., (1988)** showed the most prevalent serogroups in Egypt are O111, O126, O128, O26, O25, and O125. In Iraq, four EPEC serogroups, including O111, O86, O125, and O119, are commonly isolated from cheese samples (**Abbar, 1988**). In this study, EPEC serogroups were detected as O119 serogroup. Similar to our results, **Abbar(1988)** reported that O119 is one of the prevalent EPEC serogroups in cheese. In Australia, O119 serogroup was isolated from 161 patients with diarrhea symptoms due to the consumption of soft cheese (**Crossly *et al.*, 1995).**

In another study in the west of Iran, ***E. coli*** was isolated from 51% of domestic Iranian soft cheese samples (**Shridhar *et al.*, 2004).** It appears that the rate of contamination of soft cheese with ***E. coli*** varies in different parts of Iran.

According to the results of this study, 19.48% of isolated ***E. coli*** strains from domestic soft cheese belong to EPEC strains.

3-Sensitivity test: Table (6)

The best antibiotics for the treatment of colibacillosis infections in animals and humans were: Ciprofloxacin, Enrofloxacin, Oxytetracycline respectively to ovoid the antimicrobial resistance. The presence of antimicrobial resistance with virulence genes among STEC strains is worrisome. Therefore, when the relationship between virulence genes and antimicrobial resistance occurs the antimicrobial resistance might be selected through the antimicrobial use, and as a result, the virulence genes would be selected as well.

It is evident from the table before that ***E. coli*** virulence genes of pathogenicity that causes public hazard on cheeses consumers even cause could be discussed as follows: Table (2,7) :

1- ***Stx1*** gene It is a Shiga toxin potent exocytotic and causes severe illness:

Its presence in our isolates by PCR was zero % and not found in all ***E.coli*** isolates Nearly similar findings were reported by **Taha *et al.*,(2019*)*** while higher incidences findings were reported by **Ibrahim *et al .,(2019),* Sabrey *et al.*,(2008), Hussien *et* al., (2019), El-hadidy and Mohamed (2012), Embarks *et al.*, (2016), Cholesteric *et* al.,(2017).**

2- ***Eae A*** gene it an intimin secretion gene that enhance attachment and colonization:

Its presence in our isolates by PCR was 28.5 % of ***E. coli*** isolates: while lower incidences findings were reported by **Ibrahim *et al., (2019),* Sabrey *et al.,***

***(2008),* Hussien *et al., (2019*), El-hadidy and Mohamed *(2012),* Chalechtori *et al.*, (2017).**

3- ***Fim H gene*** it is responsible for biofilm formation and enhance infection of Uropathogenic *Escherichia coli* and the structural rule of type 1 fimbriae and responsible of about 92 % of the pathogenicity and diseased cases in human patients suffering from Urinary tract infections: Its presence in our isolates by

PCR was 100 % of *E. coli* isolates: similar results and findings were reported by **Garofalo *et al.*, *(2007)*** while lower incidences findings were reported by**, Watts *et al.*, *(2010),* Mladin *et al.*, (2009), Arabi *et al.*, (2012), Tarchouna *et al.*, (2013), Sabrey *et al.*, (2008), Zohreh *et al.*, (2014), Ibrahim *et al.*, (2019).**

the current study supports the finding that raw milk and various milk products can be regarded as critical sources of pathogenic *E. coli* This explains the need of strict monitoring and surveillance for effective measures of hygiene and sanitary practice during the production of milk and various milk products.

CONCLUSION AND RECOMMENDATIONS

6-Conclusion and Recommendations

Current Study Supports The Finding That Soft Cheese Can Be Regarded As Critical Source Of Pathogenic *E. coli*. This Explains The Need Of Strict Monitoring And Surveillance For Effective Measures Of Hygiene And Sanitary Practices During the Production Of Soft Cheeses (Kariesh, Domiatti, Tallaga). The obtained results in this work give pieces information that some of the examined soft cheese like Kareish Cheese, Damietta cheese ,and Tallaga Cheese Samples Sold in the Gharbia Governorate Were Of Poor Microbiological Quality And That Indicate Inadequate Hygienic Measures Adopted During Their Production, Handling, Processing, Packaging And Distribution Of The Examined Soft Cheeses Products.

Contamination Of The Examined Kareish Cheese, Damietta cheese ,and Tallaga Cheese Samples With Pathogenic Microorganism with *E.coli* is a reliable index of fecal pollution, poor personal hygiene, constitute a public health hazard can range from self-limited watery diarrhea to life-threatening manifestations such Hemorrhagic Colitis, Hemolytic Uremic Syndrome, Thrombocytopenic Purpura, Meningoencephalitis and may lead to death and may lead to food poisoning and undesirable changes of the products that render them unfit for human consumption that resulted in Health And Economic Losses.

Therefore, to SafeGuard Consumers From Being Infected Preventing The Sailing Of This Types Of Soft Cheeses From Excellent Surveillances On The Land Of The Targeted Zones In The West Territories Otherwise Produced This Types Of Cheeses Under The Veterinary Supervisions And Applied The Good Hygienic Practices During The Cheeses Manufacturing Stages After Ensuring That The Milk Of No Microbial Loads.

Stipulated the cheeses packages must have all the details of manufacturers and certificates of hygienic practices certificates and must be of the current year.

In Conclusion, It Deems Necessary That Concerned Authorities Should Impose Regulations And Bacteriological Standards For Control Of Soft Cheeses Production And Handling Not Only For Detection Of Errors And Defects But To Ensures That The Errors Are Corrected And Defects Are Not Repeated And Ensure A maximum Of Safety To The Consumers.

SUMMARY

7- Summary

This study was carried out to investigate isolations and serological identification of pathological pathotypes of *Escherichia coli* from Kariesh, Tillage, and Damietta types of cheeses in El _Gharbia governorate; as well as molecular identifications of some virulence genes from the isolated strains. One hundred and fifty samples of soft cheese, including 50 of each Kareish cheese, Damietta cheese, and Tallga cheese were collected from supermarkets, dairy shops, and street vendors at El- Gharbia governorate.

Microbiological examination of the examined samples revealed that *E. coli* could be detected in 54%, 42%, and 32% of examined Kareish cheese, Damietta cheese , and Tallage cheese samples, respectively. Serological typing of isolated strains of *E. coli* proved that they belong to Six Serotypes : O1119, O111, O86, O18, O128, and O124).

The Serologically Identified Pathotypes Of *E. coli Isolates* From Some Types Of Soft Cheese Were: (O119) **(EPEC)** Enteropathogenic *Escherichia coli*, (111)

(EHEC) Enterohemorrhagic *Escherichia coli*,(O86:) **)** Enteropathogenic *Escherichia coli*, (O 18) (**Ex PEC)** Extraintestinal pathogenic *Escherichia coli*,**(O128) (Atypical EPEC) Atypical EPEC** *Escherichia coli*, (**O124) (EIEC**) Entero Invasive Escherichia coli. *E. coli* infections from contaminated cheeses surely constitute the publics hazards of living creatures especially in humans likes meningeal encephalitis, pyelonephritis, hepatitis, pneumonitis, pleurisy, pericarditis, peritonitis, osteomyelitis an appendicitis.

And even causes outbreaks and death of the consumers because cheeses eating without further heating for pasteurizations.

On the other hand, molecular identifications of some virulence genes from the isolated strains. *stx1* gene was negative in all examind sample ; e*ae A* gene were detected in 28.5% of examind sample, while *fim H* gene was discovered in all samples.

In conclusion, isolation of EPEC serogroups from domestic soft cheese represents a potential, as well as an indication, of the presence of other enteropathogenesis. Although recent studies on virulence factors indicate that not all EPEC strains can attaching /effacing lesion, it is however believed that a high prevalence of contamination with EPEC strains increases the risk of infection for children, due to the consumption of domestic soft cheese. It seems that further epidemiological investigations are needed to reveal the importance of contamination in domestic soft cheese in this area of Egypt.

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ARABIC SUMMARY

الملخص العربى

أجريت هذه الدراسة لمعرفة مدى تواجد ميكروب الاشريشيا القولونية وعزلها وتصنيفها من عينات الجبن القريش والدمياطى والثلاجة فى محافظة الغربية وكذلك التصنيف الجزيئى لبعض جينات الدراوة الموجودة بالعترات المعزولة حيث تم تجميع ١٥٠ عينة من الأجبان الطرية بمعدل ٥٠ عينة من كل من الجبن القريش والجبن الدمياطى وجبن الثلاجة والتى تباع بالسوبر ماركت ومحلات الألبان والباعة الجائلين في محافظة الغربية.

أظهر الفحص الميكروبيولوجي للعينات التي تم فحصها أن ميكروب الإشريشيا القولونية قد تم اكتشافها في ٥٤٪ و ٤٢٪ و ٣٢٪ من عينات الجبن القريش والجبن الدمياطى وجبن التلاجة التي تم فحصها على التوالي. وأثبت التصنيف السيرولوجى للعترات المعزولة من ميكروب الإشريشيا القولونية أنها تنتمي إلى ستة أنماط سيرولوجية هى :

O119، O86،O18،O128 ، O124 ،O111

وتم عمل إختبار حساسية على المزارع البكتيرية حيث وجد إن المضادات الحيوية وهى بالترتيب : السيبروفلوكساسين والإنروفلوكساسين والأوكسى تترسيكلين تؤثر وتثبط نمو المعزولات السابقة من ناحية أخرى ، تم عمل الدراسات الجزيئية لبعض الجينات من السلالات المعزولة(سبع معزولات) بإنزيم البلمرة المتسلسل كان الجين stx1 سلبيًا في جميع عينات الفحص ؛ تم اكتشاف جين eae A في ٢٨.٥٪ من عينة الفحص ، بينما تم اكتشاف جين fim H في جميع العينات.

الملخص العربى

نبذة عن حياة الباحث

- ولد الباحث فى التاسع من سبتمبر ١٩٨١ بمحافظة البحيرة بمصر.

- أنهى تعليمه الابتدائي والإعدادي عام ١٩٩٣ في المملكة العربية السعودية.

- أنهى تعليمه الثانوي في مدرسة الدلجمون الثانوية بمحافظة الغربية - مصر - والتي تخرج منها عا ١٩٩٩.

- حصل على بكالوريوس العلوم الطبية البيطرية من كلية الطب البيطري ، جامعة كفر الشيح ، عام ٢٠٠٣ بتقدير عام جيد جدا.وكان ترتيبه السابع.

- يعمل بمعهد بحوث الصحة الحيوانية بفرع طنطا .

جامعة بنها
كلية الطب البيطرى بمشتهر
قسم البكتريا و المناعة و الفطريات

قرار لجنة الحكم والمناقشة

قررت لجنة الحكم والمناقشة المكونة من السادة الآتية اسماؤهم بعد جلستها اليوم الموافق ٢٠٢٠/٩/٢٧ منح ط.ب / **محمد محمود محمد يوسف وهيبه** درجة الماجستير في العلوم الطبية البيطرية تخصص البكتريولوجيا والمناعة والفطريات من كلية الطب البيطري بمشتهر جامعة بنها .

أعضاء لجنة الحكم والمناقشة :-

أ.د/ أحمد عفيفي عبد الغفار معروف	**رئيس خارجي**
رئيس بحوث معهد بحوث صحة الحيوان ببنها	.....
أ.د/ أشرف عواد عبدالتواب	**مشرف ومحكم**
استاذ ورئيس قسم البكتيريولوجيا والمناعة والفطريات	.....
أ.د / أمل مصطفي عيد	**مشرف ومحكم**
رئيس بحوث صحة أغذية معهد بحوث صحة الحيوان فرع طنطا	.....
أ.د.م / فاطمة إبراهيم الحوفي	**محكم**
أستاذ مساعد البكتريولوجيا والمناعة والفطريات	.....

كلية معتمدة ٢٠١٣

جامعة بنها
كلية الطب البيطرى
قسم البكتيريولوجى والمناعة والفطريات

تواجد ميكروبات التسمم الغذائى فى بعض أنواع الجبن الطرى فى السوق المحلى

رسالة مقدمة من

محمد محمود محمد يوسف وهيبه

بكالوريوس الطب البيطرى بكفرالشيخ جامعة طنطا عام ٢٠٠٣

لنيل درجة الماجيستير فى العلوم الطبية البيطرية

قسم البكتيريولوجى والمناعة والفطريات

تحت إشراف

الأستاذ الدكتور

أشرف عواد عبد التواب

أستاذ ورئيس قسم البكتيريا والمناعة والفطريات

كلية الطب البيطرى

جامعة بنها

الأستاذة الدكتورة

أمل مصطفى عيد

رئيس بحوث من مركز البحوث الزراعية بالقاهرة

معهد بحوث صحة الحيوان المصرى/ فرع طنطا

٢٠٢١م